NeoBiota 76: 109–133 (2022)
doi: 10.3897/neobiota.76.82776
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Risk of invasiveness of non-native fishes in the South Caucasus biodiversity and geopolitical hotspot

Levan Mumladze[1], Tatia Kuljanishvili[2], Bella Japoshvili[1], Giorgi Epitashvili[1], Lukáš Kalous[2], Lorenzo Vilizzi[3], Marina Piria[3,4]

1 *Institute of Zoology, Ilia State University, Tbilisi 0162, Georgia* **2** *Department of Zoology and Fisheries, Faculty of Agrobiology, Food, and Natural Resources, Czech University of Life Sciences, 165 00 Prague, Czech Republic* **3** *Department of Ecology and Vertebrate Zoology, Faculty of Biology and Environmental Protection, University of Lodz, 90-237 Lodz, Poland* **4** *University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, 10000 Zagreb, Croatia*

Corresponding author: Lorenzo Vilizzi (lorenzo.vilizzi@gmail.com)

Academic editor: Grzegorz Zięba | Received 26 February 2022 | Accepted 30 May 2022 | Published 3 October 2022

Citation: Mumladze L, Kuljanishvili T, Japoshvili B, Epitashvili G, Kalous L, Vilizzi L, Piria M (2022) Risk of invasiveness of non-native fishes in the South Caucasus biodiversity and geopolitical hotspot. In: Giannetto D, Piria M, Tarkan AS, Zięba G (Eds) Recent advancements in the risk screening of freshwater and terrestrial non-native species. NeoBiota 76: 109–133. https://doi.org/10.3897/neobiota.76.82776

Abstract

Aquatic invasions are one of the major threats for freshwater ecosystems. However, in developing countries, knowledge of biological invasions, essential for the implementation of appropriate legislation, is often limited if not entirely lacking. In this regard, the identification of potentially invasive non-native species by risk screening, followed by a full risk assessment of the species ranked as higher risk, enables decision-makers to be informed about the extent of the threats posed to the recipient (risk assessment) area. In this study, 32 non-native extant and horizon fish species were screened for their risk of invasiveness under current and predicted climate conditions for the South Caucasus – a biodiversity and geopolitical hotspot that includes the countries of Armenia, Azerbaijan and Georgia. Overall, the number of very high-risk species increased from four (12.5%) under current climate conditions to 12 (37.5%) under predicted climate conditions. The highest-risk species under both conditions included the already established gibel carp *Carassius gibelio* and topmouth gudgeon *Pseudorasbora parva*, the locally translocated pikeperch *Sander lucioperca* and the horizon North African catfish *Clarias gariepinus*. Under predicted climate conditions, a very high risk of invasiveness was predicted also for the translocated three-spined stickleback *Gasterosteus aculeatus* and Eurasian perch *Perca fluviatilis*, for the already established eastern mosquitofish *Gambusia holbrooki*, ruffe *Gymnocephalus cernua*, sharpbelly *Hemiculter leucisculus* and Nile tilapia *Orechromis niloticus*, and for the horizon pumpkinseed *Lepomis gibbosus* and largemouth

bass *Micropterus salmoides*. Future research on the non-native species in the South Caucasus should be conducted both country- and region-wide and should account not only for the high biodiversity, but also for the critical geopolitical situation affecting the study area.

Keywords
Aquatic invasions, AS-ISK, Black Sea, Caspian Sea, climate change, extant, horizon

Introduction

Biological invasions are a major threat to global biodiversity and pose a considerable challenge for human well-being (Mazza et al. 2014; Shackleton et al. 2018). Protecting biodiversity and maintaining ecosystem function involves the allocation of considerable financial resources due to the growing trend of invasion events and the resulting rate of pressure exerted by invasive non-native species (Seebens et al. 2017; Diagne et al. 2020). Additionally, the management and eradication of invasive non-native species once they become established are far more demanding endeavours in terms of costs and related challenges than prevention and early detection (Simberloff et al. 2013). The latter can be achieved through the establishment of country-based regulations to control species translocations and introductions within/amongst countries, supported by actions for eradication (Simberloff et al. 2013). Yet, concerted efforts to mitigate the non-native species invasion process and promote management actions pose overall a challenge at the global scale (Genovesi et al. 2013; Tittensor et al. 2014; CBD 2018).

One of the main reasons hindering effective national and cross-national strategic plans against invasive non-native species is the absence of quality biological data for several countries (Latombe et al. 2017). This is especially true of most developing countries that lack an exhaustive list of invasive non-native species, do not have monitoring capacities and/or have not yet adopted strategies for dealing with such species, which are usually identified long after their entry and establishment in the recipient area (Early et al. 2016). A good example is the South Caucasus biodiversity hotspot, which comprises the countries of Armenia, Azerbaijan and Georgia (Mittermeier et al. 2004). Despite preliminary efforts, there are currently no effective nation-wide initiatives in these countries to provide an inventory of non-native species, nor are there any related monitoring programmes in place. In fact, only incidental academic studies have so far provided some authoritative, albeit in most cases still partial, inventories of non-native plants (Kikodze et al. 2010; Fayvush and Tamanyan 2014; Sharabidze et al. 2018; Abdiyeva 2019), molluscs (Mumladze et al. 2019), insects (Aleksidze et al. 2021) and fishes (Kuljanishvili et al. 2021b). Yet, none of the aforementioned inventories (except for fishes) is complete or up-to-date enough to be useful at the national or cross-national level. Moreover, the impacts of invasive non-native species on the biodiversity and ecosystems of the South Caucasus have not yet been evaluated even for a single species, despite some of the non-native species therein having already caused environmental and economic losses (Diagne et al. 2020).

The South Caucasus is widely recognised as a biodiversity hotspot characterised by a great diversity of landscapes and climate zones that shelter a highly diverse plant and animal biota. Freshwater biodiversity is the most understudied ecological aspect of the South Caucasus (Mumladze et al. 2020), whose watercourses are exploited for hydropower production (Japoshvili et al. 2021) and secondarily for drinking water uptake, fisheries, irrigation and recreational activities. However, the potential threats faced by the freshwater biodiversity and ecosystems of the South Caucasus have not yet been evaluated, hence remain overall poorly understood. Only in a recent study have the effects of existing and planned hydropower plants on the connectivity of fish communities in Georgia been investigated in some detail (Japoshvili et al. 2021). Additionally, in another recent study, an attempt has been made to summarise the diversity, distribution and introduction history of non-native fishes in the South Caucasus based on a literature review and social-media data (Kuljanishvili et al. 2021b).

To understand the potential risk of invasiveness posed by non-native fishes in the South Caucasus, the aims of the present study were to: (i) screen both extant and horizon species and (ii) discuss the resulting species-specific risk ranks of invasiveness also within the current geopolitical situation affecting the study area with a view to implementing future legislation. Notably, this study represents the first risk screening for the South Caucasus and Georgia in particular. It is anticipated that the outcomes of this study will provide for an important step forward in the understanding of the impacts and related risks of environmental/economic losses caused by invasive non-native fishes in this biodiversity and geopolitical hotspot.

Methods

Risk assessment area

The South Caucasus (hereafter, also the 'risk assessment area') is located south of the Great Caucasus mountain range and stretches across the Black and Caspian seas with 80% of its area belonging to the Kura-Aras drainage basin (Caspian Sea Basin) shared with Turkey and Iran and the remaining 20% (western part) to the Black Sea Basin (Fig. 1). The South Caucasus is politically subdivided into the three independent countries of Armenia, Azerbaijan and Georgia. However, the South Caucasus is also an individually distinct geographic unit bordered by the Great Caucasus mountain range in the north (an impassable barrier for most animal species) and the Black and Caspian seas in the west and east, respectively. Whereas the southern border of the South Caucasus, albeit less distinctively identifiable, coincides with the two large rivers Kura-Aras and Chorokh, which originate from the Anatolian Plateau in Turkey. The Kura-Aras River is the longest watercourse and flows into the Caspian Sea, with most of the eastern South Caucasian rivers draining into this river's basin, except for a few short watercourses in the extreme north-east part of Azerbaijan. Unlike Armenia and Azerbaijan, in western Georgia, several small- to medium-sized rivers drain into the

Figure 1. Map of the South Caucasus (Armenia, Azerbaijan, Georgia), representing the risk assessment area, and neighbouring countries.

Black Sea and, at higher altitudes, there are several isolated mountainous lakes with their own independent basins. Amongst these, Lake Sevan in Armenia is the largest and harbours endemic taxa.

The climate of the South Caucasus is continental-mesophilic with strong local variation due to its complex topography. According to the updated Köppen-Geiger climate map (Beck et al. 2018), the warm-temperate climate types without dry season *Cfa* and *Cfb* (with warm and hot summer, respectively) are predominant in the westernmost part of the risk assessment area, which stretches along the entire eastern Black Sea coast and extends through to the Cholchis lowland. In this area, precipitation can be as high as 4000 mm annually (Adjara Region, south-western Georgia). Going further east, at higher altitudes (700–2000 m a.s.l.) the climate changes from cold with no dry season *Dfa* (but with hot summer) to *Dfb* (with warm summer) and *Dfc* (cold summer). Further east and up to the Caspian Sea, the climate becomes drier and corresponds predominantly to the *Bsk* (cold, arid steppe) and *Csa* (temperate dry and hot summer) types, with areas of cold semi-arid climate *Bwk*. In the eastern part, there is also a climate 'island' along the foothills of the southern Great Caucasus corresponding to the *Cfa* type. At the higher altitudes (above 2500 m) of the northern Great Caucasus and southern Lesser Caucasus, the climate changes sharply from cold with no dry season and cold summer (*Dfc*) to polar (*ET*).

Currently, there are 121 freshwater and anadromous fish species known from the South Caucasus (Kuljanishvili et al. 2021b) of which nearly 30% are endemic. The Kura-Aras River is the richest with at least 16 endemic species alone, though some watercourses of the Black Sea Basin also are species-rich, for example, the River Rioni, which is the last spawning ground for at least four sturgeon species (Beridze et al. 2022). Amongst

the 121 fish species, ten are currently considered established non-native, whereas an additional five species are intra-regionally translocated (Kuljanishvili et al. 2021b). However, the conservation status of these species is largely unknown and, at the time of writing, there are only 16 species listed in the IUCN as globally threatened under various conservation statuses, with the remaining species still waiting for a comprehensive assessment.

Species selection

In total, 32 freshwater fish taxa (hereafter, for simplicity 'species') were selected for risk screening in the South Caucasus (Table 1). Notably, the marine/brackish water fishes that are frequently entering the lower reaches of rivers in the risk assessment area (i.e. golden grey mullet *Chelon auratus*, leaping mullet *Chelon saliens* and flathhead grey mullet *Mugil cephalus*) or that can survive in isolated freshwater bodies (i.e. black-striped pipefish *Syngnathus abaster*) (Elanidze 1956, 1983; Kuljanishvili et al. 2021c) will also be regarded in this study as 'freshwater species'. The criteria for species selection were as follows (Table 1):

1. Translocated species (n = 8);
2. Non-native species already present in the risk assessment area (n = 14);
3. Non-native 'horizon' species established in neighbouring countries or countries of similar climate to the risk assessment area (n = 5);
4. Non-native species recorded in the risk assessment area, but in the wild (n = 5).

Selection of species based on the first three criteria was according to the most recent non-native species list published by Kuljanishvili et al. (2021b), whereas selection based on the latter criterion relied on literature resources.

Risk screening

Risk screening was undertaken using the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016b, 2021), which is available for free download at www.cefas.co.uk/nns/tools. This taxon-generic decision-support tool consists of 55 questions: the first 49 questions comprise the Basic Risk Assessment (BRA) and address the biogeography/invasion history and biology/ecology of the species under screening; the last six questions comprise the Climate Change Assessment (CCA) and require the assessor to predict how future predicted climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. For the purposes of the CCA component of the screening protocol, an increase in temperature on average by 2 °C relative to current conditions is predicted for the SCR (Hansen et al. 2010; Beck et al. 2018). Screenings were undertaken on all species by three independent assessors, i.e. the authors BJ, GE and TK (combination 3IA: Vilizzi et al. 2022).

To achieve a valid screening, the assessor must provide for each question a response, a level of confidence for the response (see below) and a justification based on

Table 1. Freshwater fish taxa (for simplicity, 'species') screened for their potential risk of invasiveness in the South Caucasus – the risk assessment area. For each species, the following information is provided: criterion (Crit.) for selection (1 = translocated species; 2 = non-native species already present in the risk assessment area; 3 = non-native 'horizon' species established in neighbouring countries or countries of similar climate to the risk assessment area; 4 = non-native species recorded in the risk assessment area, but in the wild); a priori categorisation outcome into Non-invasive or Invasive. For the a priori categorisation, the results of the related protocol (after Vilizzi et al. 2022) are indicated: (i) FishBase (www.fishbase.org); (ii) Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI: www.cabi.org/ISC) and Global Invasive Species Database (GISD: www.iucngisd.org); (iii) Invasive and Exotic Species of North America list (IESNA: www.invasive.org); (iv) Google Scholar literature search. N = no impact/threat; Y = impact/threat; '–' = absent; n.e. = not evaluated (but present in database); n.a. = not applicable.

Species name	Common name	A priori categorisation						
		Crit.	FishBase	CABI	GISD	IESNA	Google Scholar	Outcome
Ameiurus melas	black bullhead	3	Y	Y	–	–	n.a.	Invasive
Anguilla anguilla	European eel	4	N	Y	–	–	n.a.	Invasive
Carassius gibelio	gibel carp	2	Y	Y	–	–	n.a.	Invasive
Chelon auratus	golden grey mullet	1	N	–	–	–	N	Non-invasive
Chelon saliens	leaping mullet	1	N	–	–	–	N	Non-invasive
Clarias gariepinus	North African catfish	3	Y	Y	Y	–	n.a.	Invasive
Coregonus albula	vendace	2	N	Y	–	–	n.a.	Invasive
Coregonus sp.*	–	2	N	–	–	–	N	Non-invasive
Ctenopharyngodon idella	grass carp	2	Y	Y	Y	Y	n.a.	Invasive
Gambusia holbrooki	eastern mosquitofish	2	Y	Y	Y	–	n.a.	Invasive
Gasterosteus aculeatus	three-spined stickleback	1	N	–	–	–	N	Non-invasive
Gobio artvinicus	Artvin gudgeon	1	N	–	–	–	N	Non-invasive
Gymnocephalus cernua	ruffe	2	–	Y	–	–	n.a.	Invasive
Hemiculter leucisculus	sharpbelly	2	Y	N	–	–	n.a.	Invasive
Hypophthalmichthys molitrix	silver carp	2	Y	Y	Y	Y	n.a.	Invasive
Hypophthalmichthys nobilis	bighead carp	2	Y	Y	Y	Y	n.a.	Invasive
Ictalurus punctatus	channel catfish	4	Y	Y	–	–	n.a.	Invasive
Lepomis gibbosus	pumpkinseed	3	Y	N	–	–	n.a.	Invasive
Micropterus salmoides	largemouth bass	3	Y	Y	Y	–	n.a.	Invasive
Mugil cephalus	flathead grey mullet	4	N	–	–	–	N	Non-invasive
Mylopharyngodon piceus	black carp	4	Y	Y	–	Y	n.a.	Invasive
Oncorhynchus kisutch	coho salmon	4	N	–	–	Y	n.a.	Invasive
Oncorhynchus mykiss	rainbow trout	2	Y	Y	Y	Y	n.a.	Invasive
Oreochromis niloticus	Nile tilapia	2	Y	Y	Y	Y	n.a.	Invasive
Perca fluviatilis	Eurasian perch	1	Y	Y	Y	–	n.a.	Invasive
Pseudorasbora parva	topmouth gudgeon	2	Y	Y	–	–	n.a.	Invasive
Rhinogobius lindbergi	Lin's goby	2	N	–	–	–	N	Non-invasive
Salmo ischchan	Sevan trout	1	–	–	–	–	n.a.	Non-invasive
Salmo trutta	brown trout	2	Y	Y	Y	Y	n.a.	Invasive
Salvelinus fontinalis	brook trout	3	Y	Y	Y	–	n.a.	Invasive
Sander lucioperca	pikeperch	1	Y	Y	–	–	n.a.	Invasive
Syngnathus abaster	black-striped pipefish	1	N	N	–	–	N	Non-invasive

* Reference species for the a priori categorisation: European whitefish *Coregonus lavaretus*.

literature sources. The outcomes are a BRA score and a (composite) BRA+CCA score, which is obtained after adding or subtracting up to 12 points to the BRA score or leaving it unchanged in case of a CCA score equal to 0. Scores < 1 suggest that the species poses a 'low risk' of becoming invasive in the risk assessment area, whereas scores

≥ 1 indicate a 'medium risk' or a 'high risk'. The threshold (Thr) value to distinguish between medium-risk (BRA and BRA+CCA score < Thr) and high-risk (BRA and BRA+CCA score ≥ Thr) species for the risk assessment area is obtained by 'calibration' based on the Receiver Operating Characteristic (ROC) curve analysis (see Vilizzi et al. 2022). A measure of the accuracy of the calibration analysis is the area under the curve (AUC) whose values are interpreted as: 0.7 ≤ AUC < 0.8 = acceptable discriminatory power, 0.8 ≤ AUC < 0.9 = excellent, 0.9 ≤ AUC = outstanding (Hosmer et al. 2013). For the species ranked as high risk, a distinction was made in this study of the 'very high-risk' species, based on an ad hoc threshold weighted according to the range of high-risk scores obtained for the BRA and BRA+CCA. Identification of the (very) high-risk species is useful to prioritise allocation of resources in view of a full risk assessment (Copp et al. 2016a). This examines in detail the risks of: (i) introduction (entry); (ii) establishment (of one or more self-sustaining populations); (iii) dispersal (more widely within the risk assessment area, i.e. so-called secondary spread or introductions); and (iv) impacts (to native biodiversity, ecosystem function and services, and the introduction and transmission of diseases).

For the ROC curve analysis to be implemented, the species selected for screening must be categorised a priori as 'non-invasive' or 'invasive' using literature sources. The a priori categorisation of the species was implemented as per Vilizzi et al. (2022) (Table 1). Confidence levels in the responses to questions in the AS-ISK are ranked using a 1–4 scale and based on the confidence level allocated to each response, a confidence factor (CF) is obtained that ranges from a minimum of 0.25 (i.e. all 55 questions with confidence level equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Based on all 55 Qs of the AS-ISK questionnaire, the 49 Qs comprising the BRA and the six Qs comprising the CCA, the CF_{Total}, CF_{BRA} and CF_{CCA} are respectively computed (Vilizzi et al. 2022).

Implementation of the ROC curve analysis followed the protocol described in Vilizzi et al. (2022), with the true/false positive/negative outcome distinction not applied to the medium-risk species, as they can be either included or not into a full (comprehensive) risk assessment depending on priority and/or availability of financial resources. The ROC curve fitting was in two steps. Firstly, separate ROC curves were generated for each of the three independent assessors and differences amongst the resulting three AUCs were statistically tested (Mann-Whitney U-statistic, $\alpha = 0.05$; applet StAR available at http://melolab.org/star/home.php: Vergara et al. 2008). As differences between assessor-specific AUCs were not found, in the second step, a single ROC curve was generated, based on the average species-specific BRA scores of the three assessors. Following ROC analysis, the best threshold value that maximises the true positive rate and minimises the false positive rate was determined using Youden's J statistic, whereas the 'default' threshold of 1 was set to distinguish between low-risk and medium-risk species. Fitting of the ROC curve was with package pROC (Robin et al. 2011) for R x64 v.4.0.5 (R Core Team 2021) using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along with the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). Differences in CF between components (i.e. BRA and BRA+CCA) were tested with permutational ANOVA. Analysis was implemented

in PERMANOVA+ for PRIMER v.7, with normalisation of the data and using a Bray-Curtis dissimilarity measure, 9999 unrestricted permutations of the raw data and with statistical effects evaluated at $\alpha = 0.05$.

Results

There were no differences between the AUCs resulting from the three assessor-specific ROC curves (BJ vs. GE: $P = 0.912$; BJ vs. TK: $P = 0.090$; GE vs. TK: $P = 0.287$): this justified computation of one ROC curve based on the mean BRA scores for the screened species. Accordingly, the ROC curve resulted in an AUC of 0.8213 (0.6310–1.000 95% CI), which indicated that the risk screening was able to distinguish with excellent discriminatory power between invasive and non-invasive fish species for the risk assessment area. Youden's *J* provided the threshold of 18, which was used to calibrate the risk outcomes to distinguish between medium-risk and high-risk species. The AS-ISK report for the 32 screened species is provided as Suppl. material 1.

Based on the BRA outcome scores (Table 2, Fig. 2A):

- 21 (65.6%) species were ranked as high risk and eleven (34.4%) as medium risk;
- Amongst the nine species categorised a priori as non-invasive, one was a false positive (three-spined stickleback *Gasterosteus aculeatus*);
- Amongst the 23 species categorised a priori as invasive, 20 were true positives;
- Of the 11 medium-risk species, eight were a priori non-invasive and three invasive.
- Based on the BRA+CCA outcome scores, hence after accounting for climate change predictions (Table 2, Fig. 2B):
- 23 (71.9%) species were ranked as high risk, eight (25.0%) as medium risk and one (3.1%) as low risk (*Coregonus* sp.);
- Amongst the a priori non-invasive species, four were false positives (*Chelon auratus*, *Gasterosteus aculeatus*, Lin's goby *Rhinogobius lindbergi*, *Syngnathus abaster*) and one a true negative (*Coregonus* sp.);
- Amongst the a priori invasive species, 19 were true positives;
- Of the nine medium-risk species, four were a priori non-invasive and four invasive.

The highest-scoring ('top invasive') species (based on an ad hoc 'very high risk' threshold = 40) were gibel carp *Carassius gibelio*, North African catfish *Clarias gariepinus*, topmouth gudgeon *Pseudorasbora parva* and pikeperch *Sander lucioperca* for both the BRA and BRA+CCA, and eastern mosquitofish *Gambusia holbrooki*, three-spined stickleback *Gasterosteus aculeatus*, ruffe *Gymnocephalus cernua*, sharpbelly *Hemiculter leucisculus*, pumpkinseed *Lepomis gibbosus*, largemouth bass *Micropterus salmoides*, Nile tilapia *Oreochromis niloticus* and Eurasian perch *Perca fluviatilis* for the BRA+CCA only. Overall, the number of very high-risk species increased from four (12.5%) under the BRA to 12 (37.5%) under the BRA+CCA (Figs 2A and B). The CCA resulted in an increase in the BRA score (cf. CCA) for 26 species and in a decrease for the remaining six species (Table 2). Across the three assessors: differences in BRA scores ranged from

Table 2. Risk outcomes for the freshwater fish species screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the South Caucasus. For each species, the following information is provided: a priori categorisation for invasiveness (N = non-invasive; Y = invasive: see Table 1); min, max and mean Basic Risk Assessment (BRA) and BRA + Climate Change Assessment (BRA+CCA) scores with corresponding risk ranks (L = Low; M = Medium; H = High; VH = Very high, based on an ad hoc threshold = 40) and classifications (Class: FP = false positive; TN = true negative; TP = true positive; '–' = not applicable as medium risk: see text for details); difference (Delta) between mean BRA+CCA and BRA scores. Risk ranks are based on a threshold of 18 and computed as: L, with score within the interval [–20, 1[, M [1, 18[, H [18, 40[, VH [40, 68] for the BRA; L [–32, 1[; M [1, 18[, H [18, 40[, VH [40, 80] for the BRA+CCA (note the reverse bracket notation indicating in all cases an open interval).

Species name	A priori	BRA					BRA+CCA						CF		
		Score					Score								
		Min	Max	Mean	Rank	Class	Min	Max	Mean	Rank	Class	Delta	Total	BRA	CCA
Ameiurus melas	Y	26.5	35.0	31.5	H	TP	24.5	47.0	36.8	H	TP	5.3	0.70	0.71	0.61
Anguilla anguilla	Y	1.0	15.0	9.3	M	–	–5.0	17.0	8.0	M	–	–1.3	0.72	0.72	0.71
Carassius gibelio	Y	36.0	52.0	44.0	VH	TP	48.0	64.0	55.3	VH	TP	11.3	0.74	0.75	0.67
Chelon auratus	N	14.0	25.0	17.7	M	–	16.0	20.0	18.3	H	FP	0.7	0.70	0.72	0.46
Chelon saliens	N	13.0	23.0	16.7	M	–	15.0	20.0	17.3	M	–	0.7	0.65	0.68	0.42
Clarias gariepinus	Y	38.0	45.0	40.3	VH	TP	46.0	55.0	49.7	VH	TP	9.3	0.66	0.68	0.51
Coregonus albula	Y	5.0	19.5	11.2	M	–	–3.0	7.5	1.2	M	–	–10.0	0.71	0.72	0.63
Coregonus sp.	N	1.0	18.0	9.0	M	–	–7.0	6.0	–0.3	L	TN	–9.3	0.70	0.71	0.61
Ctenopharyngodon idella	Y	18.0	23.5	20.7	H	TP	14.5	31.5	24.7	H	TP	4.0	0.69	0.72	0.49
Gambusia holbrooki	Y	31.5	38.0	34.5	H	TP	37.5	48.0	43.2	VH	TP	8.7	0.70	0.72	0.51
Gasterosteus aculeatus	N	37.0	38.0	37.7	H	FP	38.0	44.0	41.0	VH	FP	3.3	0.67	0.69	0.50
Gobio artvinicus	N	5.0	14.0	8.7	M	–	7.0	15.0	12.0	M	–	3.3	0.59	0.61	0.44
Gymnocephalus cernua	Y	34.0	46.0	39.3	H	TP	46.0	58.0	50.7	VH	TP	11.3	0.63	0.65	0.50
Hemiculter leucisculus	Y	32.0	35.0	33.8	H	TP	42.0	45.0	43.8	VH	TP	10.0	0.71	0.73	0.58
Hypophthalmichthys molitrix	Y	20.5	24.0	22.8	H	TP	18.5	34.0	28.8	H	TP	6.0	0.65	0.67	0.51
Hypophthalmichthys nobilis	Y	25.5	28.0	26.8	H	TP	19.5	38.0	30.8	H	TP	4.0	0.67	0.68	0.61
Ictalurus punctatus	Y	26.0	33.0	29.0	H	TP	32.0	45.0	39.0	H	TP	10.0	0.64	0.66	0.47
Lepomis gibbosus	Y	25.5	36.0	29.8	H	TP	37.5	46.0	40.5	VH	TP	10.7	0.71	0.72	0.63
Micropterus salmoides	Y	22.0	38.5	31.2	H	TP	34.0	50.5	41.2	VH	TP	10.0	0.70	0.72	0.56
Mugil cephalus	N	6.0	22.0	11.3	M	–	12.0	18.0	14.7	M	–	3.3	0.63	0.66	0.42
Mylopharyngodon piceus	Y	20.0	24.0	22.0	H	TP	28.0	34.0	32.0	H	TP	10.0	0.66	0.69	0.44
Oncorhynchus kisutch	Y	4.0	15.0	11.2	M	–	8.0	17.0	11.8	M	–	0.7	0.64	0.66	0.50
Oncorhynchus mykiss	Y	15.0	26.5	20.2	H	TP	15.0	18.5	16.8	M	–	–3.3	0.63	0.66	0.35
Oreochromis niloticus	Y	24.0	38.0	32.7	H	TP	34.0	48.0	42.0	VH	TP	9.3	0.65	0.68	0.49
Perca fluviatilis	Y	17.0	51.0	32.0	H	TP	23.0	63.0	41.3	VH	TP	9.3	0.66	0.68	0.50
Pseudorasbora parva	Y	32.0	47.0	40.0	VH	TP	44.0	57.0	49.3	VH	TP	9.3	0.77	0.78	0.71
Rhinogobius lindbergi	N	16.0	17.5	16.5	M	–	26.0	28.0	27.2	H	FP	10.7	0.54	0.53	0.63
Salmo ischchan	N	5.0	25.0	16.7	M	–	–7.0	20.0	10.0	M	–	–6.7	0.64	0.65	0.58
Salmo trutta	Y	34.0	39.0	36.0	H	TP	31.0	40.0	36.7	H	TP	0.7	0.63	0.66	0.46
Salvelinus fontinalis	Y	17.0	33.0	23.7	H	TP	17.0	29.0	23.0	H	TP	–0.7	0.70	0.75	0.31
Sander lucioperca	Y	30.0	50.0	43.0	VH	TP	38.0	59.0	46.3	VH	TP	3.3	0.69	0.70	0.58
Syngnathus abaster	N	9.0	27.0	16.3	M	–	5.0	37.0	20.3	H	FP	4.0	0.70	0.72	0.50

0 to 34, with a mean of 11.8, a median of 11.0 and 5% and 95% CIs of 5.1 and 20.2, respectively (Fig. 3A); differences in BRA+CCA scores ranged from 4 to 35, with a mean of 16.1, a median of 13.5 and 5% and 95% CIs of 5.8 and 32.0, respectively (Fig. 3B).

In terms of confidence in responses, the mean CL_{Total} was 2.69 ± 0.03 SE, the mean CL_{BRA} 2.76 ± 0.03 SE and the mean CL_{CCA} 2.11 ± 0.07 SE (hence, indicating

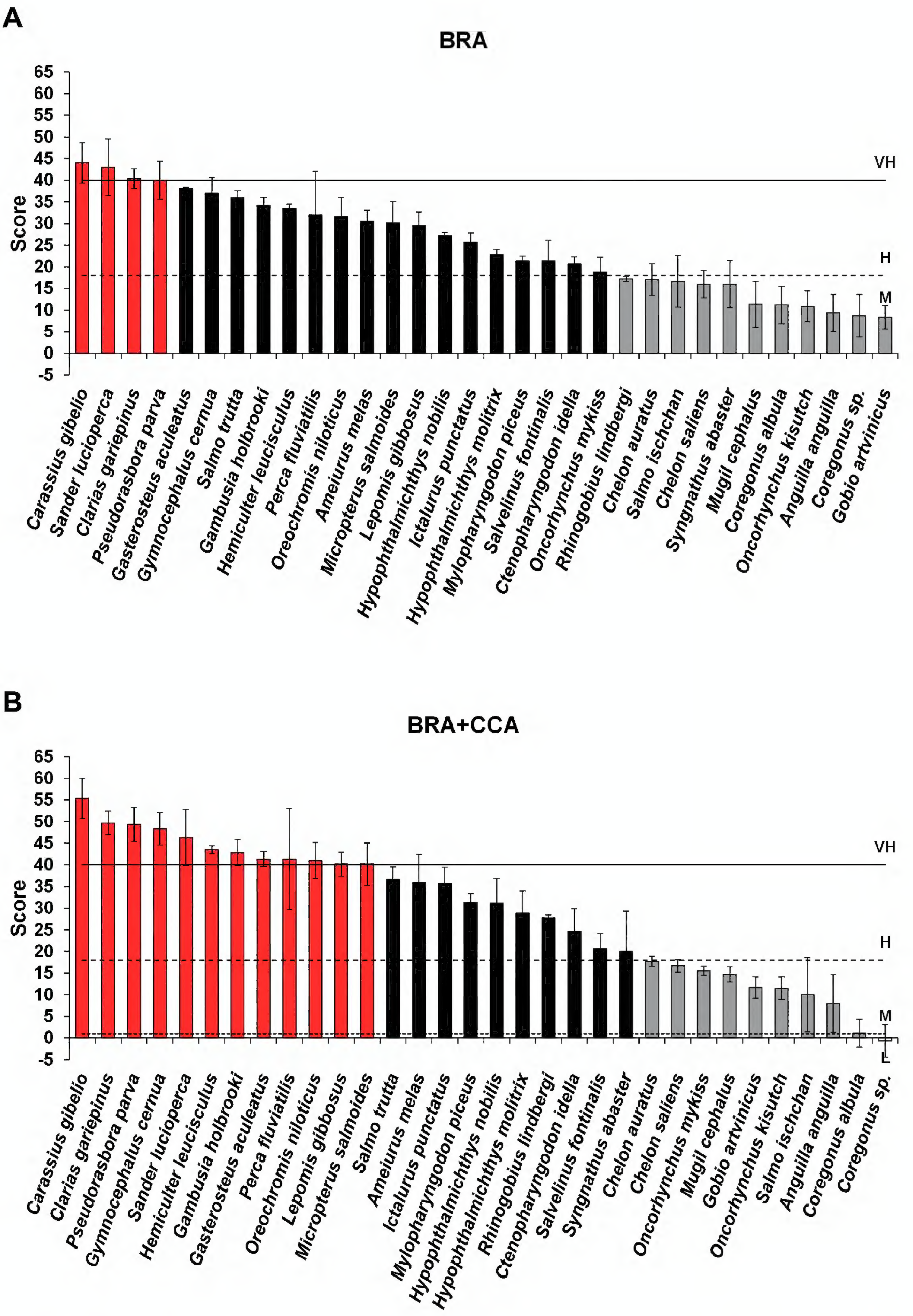

Figure 2. Aquatic Species Invasiveness Screening Kit (AS-ISK) mean outcome scores (± SE) for the species screened for the South Caucasus: **A** Basic Risk Assessment (BRA) scores **B** BRA+CCA (Climate Change Assessment) scores. Red bars = very high-risk species; Black bars = high-risk species; Grey bars = medium-risk species; White bars = low-risk (L) species. Solid line = very high-risk (VH) threshold; Hatched line = high-risk (H) threshold; Dotted line = medium-risk (M) threshold (thresholds as per Table 2).

a medium confidence level). The mean CF_{Total} was 0.673 ± 0.008 SE, the mean CF_{BRA} 0.691 ± 0.008 SE and the mean CF_{CCA} 0.527 ± 0.017 SE. Statistically, the CL_{BRA} was higher than the CL_{CCA} ($F^{\#}_{1,62}$ = 75.44, P < 0.001; # = permutational value).

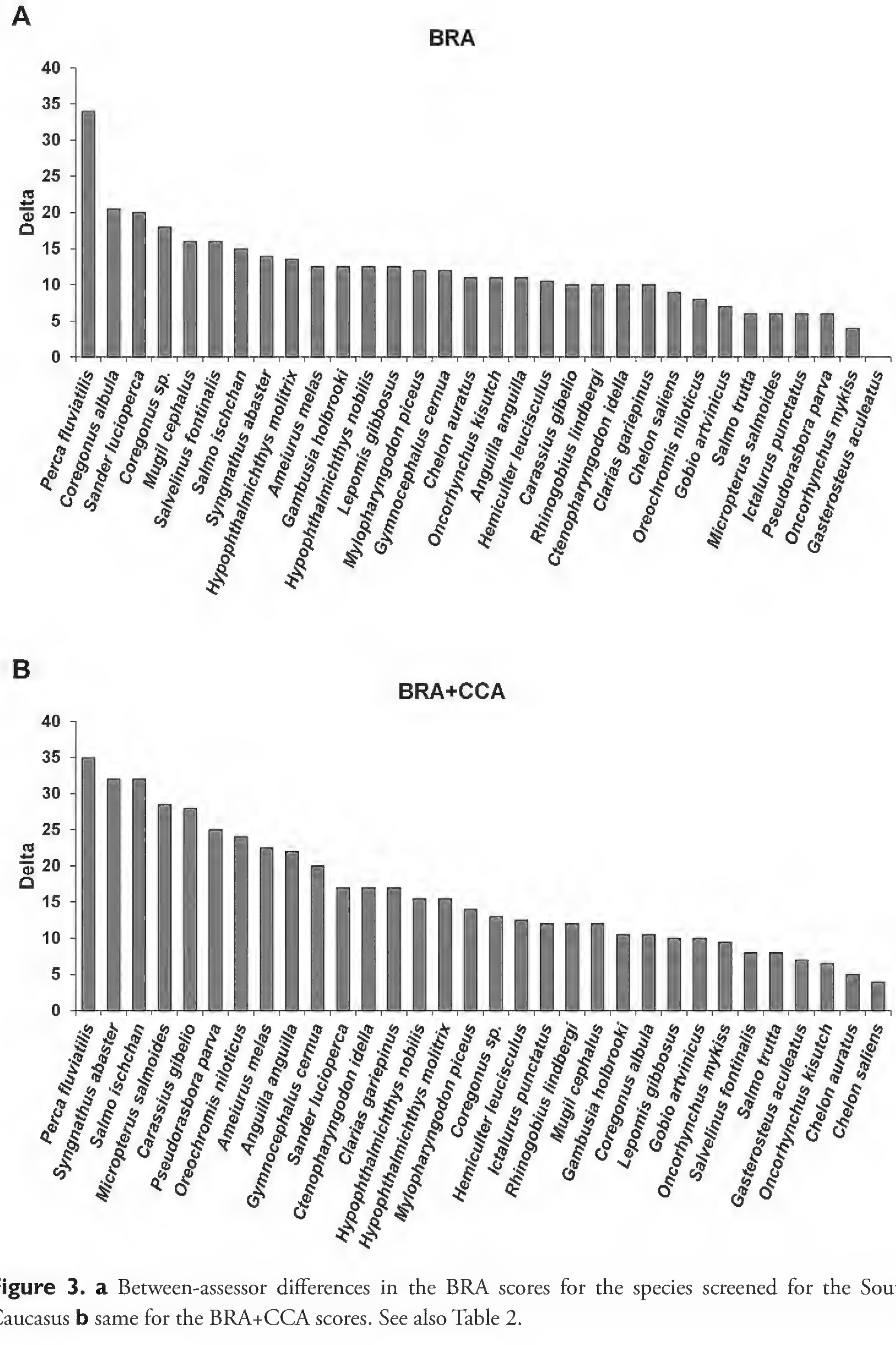

Figure 3. a Between-assessor differences in the BRA scores for the species screened for the South Caucasus **b** same for the BRA+CCA scores. See also Table 2.

Discussion

Risk outcomes

The present study, which is the first to conduct a risk screening for the South Caucasus, was able to identify with excellent discriminatory power the level of risk of invasiveness of the non-native fish species under evaluation. The calibrated threshold value (Thr = 18) in this study can, therefore, be used for future screening of additional non-native fish species in the risk assessment area, as required. Further, this threshold could be refined subject to availability of new biological data on the species screened in this study and/or additional species that may be identified as horizon or recorded in the risk assessment area by future surveys and/or based on more up-to-date climate change scenarios – this is in line with risk analysis as a dynamic, 'work-in-progress' applied field of science (see Vilizzi et al. 2021). Finally, the replication of screenings by three independent assessors in this study to account for the inherent potential bias with expert-based evaluations and the resulting lack of differences in assessor-specific AUCs has further strengthened the reliability of the species-specific risk ranks.

Amongst the screened species, 20 were ranked as carrying a high or very high risk of invasiveness under both current (BRA) and predicted climate conditions (BRA+CCA) (Table 2). These species included the false positive *Gasterosteus aculeatus*, whose high-risk ranking in the present study can, however, be justified with reference to the risk assessment area. In this respect, *G. aculeatus* is a widespread circum-arctic/temperate freshwater fish that naturally occurs in the River Danube mouth and on the Black Sea coast (Piria et al. 2018). This species has expanded its range to the Caspian Sea via the Volga-Don Channel and is now widely established along the Caspian Sea coast (eastern South Caucasus), where it actively enters the lower reaches of rivers (Ibrahimov and Mustafayev 2015). Due to its high tolerance of salinity and temperature and its reproductive and foraging characteristics (e.g. Roch et al. 2018; Candolin 2019), *G. aculeatus* can, therefore, be regarded as a high-risk species for the risk assessment area, despite its a priori non-invasive status elsewhere.

Overall, the a priori invasive species found to carry a high or very high risk of invasiveness (Table 2) may represent a threat to the native species and ecosystems of the South Caucasus. Some of these species are already established in the risk assessment area (Table 1) and, amongst these, *Carassius gibelio* and *Pseudorasbora parva* (both identified as very high risk) are already recognised as posing a serious threat (Kuljanishvili et al. 2021b). *Carassius gibelio* was introduced unintentionally (and probably several times) since the 1980s mainly as a contaminant of stockings of young-of-the-year common carp *Cyprinus carpio* in Lake Paliastomi and in the lakes of the Javakheti Plateau (Japoshvili et al. 2013; Kuljanishvili et al. 2021b). However, other species, including Chinese carps (i.e. bighead carp *Aristichthys nobilis*, *Ctenopharyngodon idella* and silver carp *Hypophthalmichthys molitrix*) and *Cyprinus carpio*, have also been intensively and mostly illegally introduced to other water bodies of the South Caucasus by local anglers for recreational purposes resulting in accidental introductions of *C. gibelio*

(Japoshvili et al. 2013; Kuljanishvili et al. 2021b). Concerning *P. parva*, this species is a typical hitchhiker without any economic value that is spreading on its own through the watercourses of the South Caucasus and is still being translocated as part of cyprinid farming practices (Gozlan et al. 2010; Kuljanishvili et al. 2021b). Overall, both species are currently the most widespread in the risk assessment area, where, in most cases, they form dense populations that dominate in abundance the local fish community (Shoniya et al. 2011; Japoshvili et al. 2013; Pipoyan and Arakelyan 2015; Kuljanishvili et al. 2021b).

A very high risk of invasiveness was also attributed to *Clarias gariepinus* and to locally translocated *Sander lucioperca*. *Clarias gariepinus* is found in neighbouring Turkey and is an invasive predator species that can easily spread once established (Ellender et al. 2015; Weyl et al. 2016). Detrimental effects of this species on the native fauna are, therefore, expected to occur in the South Caucasus as observed elsewhere (Kadye and Booth 2012; Ellender et al. 2015) and can be exacerbated by climate change (i.e. increase in temperatures) as revealed by the augmented BRA+CCA score for this species (Table 2). *Sander lucioperca* is a sought-after species with anglers that is actively translocated within the risk assessment area (Kuljanishvili et al. 2021b). This species has been reported to alter severely the invaded ecosystems in multiple ways including predation, hybridisation and disease transmission (Godard and Copp 2011). However, the species' impact on the native freshwater fauna of the risk assessment area remains unknown.

The threats posed by other established species ranked as carrying a high risk (BRA) or very high risk (BRA+CCA) of invasiveness, such as *Gambusia holbrooki*, *Gymnocephalus cernua* and *Hemiculter leucisculus* and by locally-translocated *Perca fluviatilis*, are still not clearly understood. *Gambusia holbrooki* was one of the first non-native species to be introduced in the South Caucasus for mitigation of the malaria disease (Barach 1941; Elanidze 1983). Since its introduction, this species has formed dense populations in most of the still water bodies of western Georgia and the eastern South Caucasus (Kuljanishvili et al. 2021b). The other two species *G. cernua* and *H. leucisculus* are cryptic invaders for which no invasion/establishment history exists. The former species is far more widespread in the risk assessment area than previously thought (G. Epitashvili, unpublished data), whereas the latter was first detected from the River Rioni in western Georgia in 2020 based on DNA barcoding (Epitashvili et al. 2020). *Perca fluviatilis* has been repeatedly introduced to water bodies of the South Caucasus for recreational angling, although no data are available on its introduction/establishment history, population dynamics or range of expansion (Kuljanishvili et al. 2021b). However, given its predatory lifestyle, this species can severely alter the native fish communities of the risk assessment area (review in Rowe et al. 2008).

Other species ranked as high (or very high) risk included those that are regularly stocked in the risk assessment area, but have not yet established self-sustaining populations, namely grass carp *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, bighead carp *Hypophthalmichthys nobilis*, channel catfish *Ictalurus punctatus*, black carp *Mylopharyngodon piceus*, rainbow trout *Oncorhynchus mykiss*, Nile tilapia *Orechromis niloticus* and brown trout *Salmo trutta*. Amongst these species, *O. niloticus* is currently

considered a hitchhiker in the South Caucasus, where no information on its deliberate farming is available. This species has been recorded only once in the wild (River Alazani, eastern Georgia), though no established population has been confirmed (Kuljanishvili et al. 2021a). Overall, all of the above species could pose a substantial threat to the local native ecosystems once established (e.g. Dibble and Kovalenko 2009; Martín-Torrijos et al. 2016; DeBoer et al. 2018; Faria et al. 2019), so their stocking should either be avoided altogether or strictly monitored.

The horizon species black bullhead *Ameiurus melas*, *Lepomis gibbosus*, *Micropterus salmoides* and brook trout *Salvelinus fontinalis* were also ranked as high (or very high) risk. However, neither of them has so far been recorded from the risk assessment area, although they are all well known to have expanded their range worldwide as a result of introductions for recreational and aquaculture purposes. Although a proper understanding of their impact on the invaded ecosystems is limited, these species are known to pose substantial threats to the native fish faunas (e.g. Cucherousset et al. 2008; Leunda et al. 2008; Drake 2009; Almeida et al. 2014; Copp et al. 2017).

Of the eleven species found to carry a medium risk of invasiveness (based on the BRA), vendace *Coregonus* sp., *Coregonus albula*, coho salmon *Oncorhynchus kisutch* and *Rhinogobius lindbergi* deserve some special consideration. Both *Coregonus* sp. (a putative hybrid known as *C. lavaretus sevanicus*: Dadikyan 1964, 1986) and *C. albula* have been regularly stocked in the mountainous lakes of Georgia (Javakheti Plateau) and in Lake Sevan, where they are thought to have established self-reproducing populations and are also highly valued commercially (Dadikyan 1964; Japoshvili 2012; Kuljanishvili et al. 2021b). In contrast, *O. kisutch* was released only once into the Caspian Sea in the 1980s and no further information on this species has since been available (Musayev et al. 2004). The small gobiid fish *R. lindbergi*, which is already widespread in the south-western Caspian Sea Basin (Sadeghi et al. 2019; Japoshvili et al. 2020), is not known to pose any risk to the native fauna, although this may be only a provisional expectation. This is because *R. lindbergi* resembles native gobies and occupies a similar ecological niche. Additionally, being a cryptic invader (e.g. Epitashvili et al. 2020), *R. lindbergi* may pose a threat to the native fauna; hence, it should be subject to future monitoring.

The remaining species carrying a medium risk of invasiveness are all native to the South Caucasus and translocated, except for *Mugil cephalus*. Four of these species, namely the mullets *Chelon auratus*, *Chelon saliens* and *Mugil cephalus*, as well as *Syngnathus abaster*, are primarily marine/brackish water species regularly occurring in estuaries or in the lower stretches of rivers. Mullets, which are economically valuable and naturally occur in the Black Sea, were introduced to the Caspian Sea in the early 20th century and, amongst them, *C. saliens* and *C. auratus* have established dense and abundant populations in river mouths (Bogutskaya et al. 2013). Risk screening for the latter two species has also been conducted for the neighbouring Anzali Wetland Complex (Caspian Sea Basin, Iran), with a similar medium-risk rank for *C. saliens*, but a low-risk rank for *C. auratus* (Moghaddas et al. 2021). This was mainly due to the fact that these species are known to reproduce in the Caspian Sea and to use associated estuaries and river mouths only temporarily for feeding (Coad 2017). As per *M. cephalus*, this species is currently not known to be established in the risk assessment area. However, given its

high tolerance for water temperature and salinity, *M. cephalus* could reach the Caspian Sea Basin either on its own (i.e. via the Volga-Don Channel) or by translocation for aquaculture purposes (Abo-Taleb et al. 2021). The other eurihaline translocated species *S. abaster*, which is native to the Black and Caspian sea coasts (and with a threatened status in the Caspian Sea: Kolangi-Miandare et al. 2013), was introduced into Tbilisi Reservoir in the 1980s and has since resulted in the establishment of a dense population (Kuljanishvili et al. 2021b). Although not regarded as invasive, *S. abaster* is known for its ability to establish easily in freshwater habitats and affect zooplankton communities by selective feeding (Didenko et al. 2018).

The remaining translocated species ranked as medium risk included migratory European eel *Anguilla anguilla* and the resident species Artvin gudgeon *Gobio artvinicus* and *Salmo ischchan*. *Anguilla anguilla* was recorded from the Caspian Sea Basin in 1964 (Kuljanishvili et al. 2021b) and has been reported to enter various rivers of the risk assessment area, where it occurs, however, at low densities (Abdurakhmanov 1966; Ibrahimov and Mustafayev 2015; Pipoyan 2015). *Gobio arthvinicus* was unintentionally translocated from the Black Sea Basin to the River Kura, where it has most probably spread as a hitchhiker, though it is not used either for recreational or aquaculture purposes. This species is already established in the River Kura Basin (Kuljanishvili et al. 2021b) and its distribution has expanded to other watercourses. In contrast, *S. ischchan* was introduced to water bodies of Azerbaijan and Georgia from Lake Sevan and self-sustaining populations have been reported to cause negative impacts on native Caspian trout *Salmo caspius* (Elanidze 1983; Musayev et al. 2004; Yusifov et al. 2017; Kuljanishvili et al. 2021b).

Overall, under predicted climate change, 12 species in total were ranked as very high risk (Table 2, Fig. 1B). Of these species, eight are already established in the risk assessment area of which three are translocated (i.e. *Gasterosteus aculeatus*, *Perca fluviatilis*, *Sander lucioperca*) and six introduced (i.e. *Carassius gibelio*, *Gambusia holbrooki*, *Gymnocephalus cernua*, *Hemiculter leucisculus*, *Oreochromis niloticus*, *Pseudorasbora parva*) and three are horizon (i.e. *Clarias gariepinus*, *Lepomis gibbosus*, *Micropterus salmoides*). The current lack of legislation for non-native species in the South Caucasus, hence strategies for dealing with the impacts of current invasions, is therefore an issue of even higher concern given the predicted increase in number of very high risk species because of climate change (see below).

Recommendations for future research

In this study, the South Caucasus has been treated as a distinct biogeographic unit rather than a politically defined entity at the country level, hence in line with the preferred approach to the definition of a risk assessment area (Vilizzi et al. 2022). This is because the Kura-Aras Basin is shared amongst all the South Caucasus countries of Armenia, Azerbaijan and Georgia, making establishment of invasive non-native species in any of them likely to result in future potential ecological impacts for all three countries. In addition, the Kura-Aras Basin is also shared between Turkey and the South Caucasus and the same is true for the River Chorokhi between Turkey and western Georgia.

Consequently, any evaluation of the impact of potentially invasive non-native species and the establishment of regulations for their introduction and management should ideally be agreed upon and implemented across all of the South Caucasus countries (if not beyond: cf. Turkey), rather than independently for each of them. Unfortunately, the South Caucasus biodiversity hotspot is also a 'geopolitical hotspot' subject to permanent military tensions, which are likely to be exacerbated in recent times (Muradov 2022). For this reason, it is hardly possible to communicate and discuss the outcomes of environmental issues not only between countries (i.e. Armenia vs Azerbaijan), but also at the country level (e.g. 20% of the territory of Georgia is inaccessible due to Russian occupation), with the result that these long-unresolved political tensions are further aggravating the extent of non-native species management plans across the South Caucasus.

In the European Union, policies, legislation and management approaches have been developed to address the issue of non-native species, based on Regulation (EU) no. 1143/2014 of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species (Piria et al. 2017, 2021). However, countries outside of the EU do not have an obligation to follow these rules and usually lack national legislation, which represents an additional problem for non-native species management (Piria et al. 2021). Whilst there is no region-wide agreement/management policy related to non-native aquatic species, there is also a major lack of relevant national-level legislation in the three South Caucasus countries (Kuljanishvili et al. 2021b). These all are parties to the Convention on Biological Diversity (CBD: https://www.cbd.int/) and Georgia is, in addition, a party to two other conventions, namely the Convention on the Protection of the Black Sea Against Pollution (http://www.blacksea-commission.org/) and the Convention for the Control and Management of Ships' Ballast Water and Sediments (https://www.imo.org/en). Within these Conventions (and particularly under the CBD), the South Caucasus countries are regularly developing National Biodiversity Strategy and Action Plans (https://www.cbd.int/nbsap/) in which the lack of data/infrastructure dealing with invasive non-native species is being emphasised and relevant targets (e.g. development of invasive non-native species lists, identification of introduction pathways, evaluation of impacts, implementation of legislative measures) are being proposed. However, none of the targets related to (freshwater) non-native species has so far been fulfilled (see, for example, the fifth national reports for Armenia, Azerbaijan and Georgia: https://www.cbd.int/).

Overall, to date, none of the South Caucasus countries has achieved a clear understanding of non-native species management within a national legislation plan (Kuljanishvili et al. 2021b), so that the import/farming of aquatic non-native species for recreational/aquaculture purposes is simply allowed under permission of governmental bodies. In fact, there is no legislative means for banning any particular aquatic non-native species (including highly invasive ones), checking for hitchhikers or restricting translocations. It is, therefore, strongly advocated that both country- and region-wide (i.e. South Caucasus) strategies, legislation acts and related actions for freshwater non-native species should be urgently developed. In this respect, it is recommended that such a strategy should adopt the following overarching conceptual goals:

1. Full risk assessment of any potentially invasive species should focus on those ranked as high risk (or very high risk, depending on availability of resources). Thus, whenever possible, a comprehensive risk screening, as achieved in this study, should be conducted and species-specific risk-rank outcomes presented to decision-makers. In this study, 12 very high-risk species in total (after accounting for climate change predictions) were identified that should be prioritised for follow-up full risk assessment.
2. Knowledge gap analysis and improvement of the legal basis for species introductions related to aquaculture/game fisheries and the pet trade. Ideally, this should be jointly agreed upon by the SCR countries to be fully effective.
3. Early detection and communication of freshwater non-native species is a process already under way, with researchers publishing results about new introductions of potentially invasive non-native species and citizen science platforms regularly receiving data from the general public on the identification of new non-native species. However, data and knowledge developing over time must be standardised in order to be rapidly communicated to stakeholders and decision-makers. In addition, adequate measures should be taken to enhance data collection from all potential sources. For instance, there is currently no information on non-native species available from local markets.
4. Continuous development of an in-depth monitoring scheme (including infrastructure for data collection based on fieldwork, barcoding/metabarcoding approaches, data management and presentation). This is a critical step to understand the history of non-native species colonisation and the accompanying processes related to community perception, including associated costs for damage/mitigation.
5. Prevention of introductions (cf. 'blacklists' of species). Since there is a large amount of data on freshwater non-native species worldwide, it would be straightforward to develop a list of potentially invasive non-native species for the South Caucasus (see Roy et al. 2019). In this regard, risk screening could help further refinement of the taxa list and via a similar exercise given in the present study. Control of the introduction of the most (if not all) threatening species could then be implemented by the local governments. As such practice already exists elsewhere (Essl et al. 2011; Gederaas et al. 2012; Poeta et al. 2017; Battisti et al. 2019), it should be discussed and adopted also within the countries of the South Caucasus.
6. Impact assessment research, including that related to already established invasive non-native species. Currently, there is no evaluation of the economic/environmental costs related to freshwater non-native species, nor for terrestrial ones. This makes it difficult to assess the effects of non-native species on local communities and to manage in an optimal way available resources to prevent/mitigate non-native species introductions. The results of this study can, therefore, be used to prioritise the list of fish species in the South Caucasus to be evaluated for impact assessment.

Acknowledgements

LK was partially supported by the Technology Agency of the Czech Republic under project "DivLand" (SS02030018) and IRP MSMT CZU 60460709. MP was sup-

ported by the EIFAAC Project “Management/Threat of Aquatic Invasive Species in Europe". LM, BJ and GE were supported, and the publication cost were covered, by the government subsidised grant "Current status and conservation of fauna of Georgia" implemented at the Institute of Zoology of Ilia State University.

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Supplementary material 1

Combined AS-ISK report including the 96 screenings for the 32 fish species screened for the South Caucasus

Authors: Levan Mumladze, Tatia Kuljanishvili, Bella Japoshvili, Giorgi Epitashvili, Lukáš Kalous, Lorenzo Vilizzi, Marina Piria

Data type: Pdf file

Explanation note: Combined AS-ISK report including the 96 screenings for the 32 fish species screened for the South Caucasus region.

Link: https://doi.org/10.3897/neobiota.76.82776.suppl1